30 minutes. After fixation, the above sample was washed once with PBS and blocked with 1% bovine serum albumin (Thermo Fisher Scientific, Inc.) at room temperature for 20 minutes. After blocking, the sample was washed once with PBS, and a mixture of anti-βIII tubulin antibody (Sigma-Aldrich Co., LLC) and anti-tyrosine hydroxylase antibody (Abeam plc), each of which was diluted 200-fold with PBS, was added thereto as primary antibodies and then incubated overnight at 4° C.

[0221] Subsequently, the sample was washed three times with PBS, and a mixture of APC-labeled goat anti-mouse IgG (H+L) antibody (Thermo Fisher Scientific, Inc.) and Alexafluoro 594-labeled goat anti-rabbit IgG (H+L) antibody (Thermo Fisher Scientific, Inc.), each of which was diluted 500-fold with PBS, was added thereto as secondary antibodies, incubated at room temperature for 1 hour, and then washed twice with PBS.

[0222] Subsequently, a cover glass was placed on the sample, sealing was performed using a sealing solution (product name "ProLong Diamond Antifade Mountain", Thermo Fisher Scientific, Inc.), and the sealed sample was observed under a fluorescence microscope.

[0223] FIGS. **20**A and **20**B are fluorescence photomicrographs that show the observation results of the fluorescence of βIII tubulin, which is a neural skeleton of the obtained neural circuit model, and the fluorescence of tyrosine hydroxylase, which is expressed only in PC12 cells. FIG. **20**A is a fluorescence photomicrograph of a neural circuit model in which nerve cells were arranged in the pattern illustrated in FIG. **19**A, and FIG. **20**B is a fluorescence photomicrograph of a neural circuit model in which nerve cells were arranged in the pattern illustrated in FIG. **19**B.

[0224] As a result, the fluorescence of βIII tubulin was observed in the entire cell arrangement region in the neural circuit model in which the nerve cells were arranged in any pattern. It was also confirmed that the neurite grows. In addition, it was confirmed that the fluorescence of tyrosine hydroxylase was observed only in the region in which PC12 cells are arranged and the pattern of the cell arrangement was maintained.

Experimental Example 9

[0225] (Improvement of Drying Suppression Step)

[0226] Cells were seeded on a dried substrate, and the suppression of drying of the liquid pool and the occurrence of cell colonization were evaluated. A slide glass was used as the substrate. As cells, PC12 cells, which are a cell line derived from rat pheochromocytoma, were used. Cell seeding was performed by an inkjet method.

[0227] <<Preparation of Cell Ink>>

[0228] 5 mL of a serum-free medium containing the green fluorescent dye was added to the dish of the cultured PC12 cells, and the cells were cultured in an incubator (KM-CC17RU2, manufactured by Panasonic Corporation, in an environment of 37° C. and 5% by volume CO_2) for 30 minutes. Thereafter, the cells were detached from the dish by trypsin treatment to obtain a cell suspension. Subsequently, the number of cells was measured using Nucleo Counter NC-3000 (trade name, manufactured by ChemoMetec) using some of the cell suspension.

[0229] As the dispersion medium for cell ink, PBS (−) supplemented with 0.5% by mass of glycerin (molecular biology grade, manufactured by FUJIFILM Wako Pure Chemical Corporation) as a cell-drying inhibitor was used. PC12 cells were dispersed in the dispersion medium such that the concentration thereof was 3×10^6 cells/mL, thereby obtaining the cell ink.

[0230] <<Cell Ejection>>

[0231] The liquid chamber of the cell ejection head of the device in FIG. **4** was filled with the cell ink. Subsequently, liquid droplets of the cell ink were ejected onto the substrate, and liquid pools were arranged. The diameter per one liquid pool was about 400 μm. In addition, the liquid pools arranged on the substrate contained about 100 cells per one liquid pool, and the cell density in the liquid pool was 8×10^4 cells/cm^2.

[0232] FIG. **21**A is a photomicrograph of a liquid pool coated with biocompatible oil (Oil for Embryo Culture, manufactured by Fujifilm Wako Pure Chemical Corporation) immediately after the cell ink was ejected and prevented from drying. Even in a case of leaving to stand at 37° C. for 60 minutes, a small amount of liquid pool did not dry when being coated with oil, and the cells in the liquid droplet sedimented and could temporarily adhere onto the substrate to form a cell aggregate. This method can be applied not only to PC12 cells but also to many kinds of cells.

[0233] Then, the oil was gently removed, and a medium was gently added. FIG. **21**B is a photomicrograph taken immediately after adding the medium. Since the amount of the liquid pool is very small, the liquid pool dries in a few minutes in a normal laboratory environment, and the volume of the liquid pool is reduced by 90% or more.

[0234] It was confirmed that cell sedimentation and temporary adhesion can be stably performed by a method of coating a liquid pool with oil or the like even in a case where a dried substrate is used, in addition to the method for incubating cells to stand in a high-humidity environment as in Experimental Examples 1 and 2 and the method for forming a liquid pool on a wetted substrate as in Experimental Examples 3 to 8.

[0235] Subsequently, the number of cells per one liquid pool and the presence or absence of the drying suppression treatment for the liquid pool on the dried substrate were changed variously according to the combinations shown in Table 2 below to produce each neural circuit model of Reference Examples 1 to 4, which was subsequently evaluated. A 35 mm dish was used as the substrate. As the nerve cells, GABAergic nerve cells derived from human iPS cells (manufactured by Elixirgen Scientific, Inc.) were used.

[0236] In Table 2 below, "Suppression of drying of liquid pool" indicates the presence or absence and the kind of the drying-suppressing step for the liquid pools arranged on the substrate, and "Maintenance of shape of liquid pool" indicates whether or not the shape of the liquid pool was maintained after being left to stand at 37° C. for 60 minutes. Other items are the same as those shown in Table 1 above. The evaluation criteria for "Maintenance of shape of liquid pool" were as follows.

[0237] +: The shape of the liquid pool was maintained.

[0238] −: 80% or more of the liquid pool dried, and cell death was observed.